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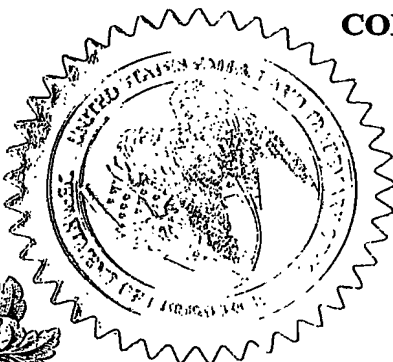
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<b>TITLE OF THE INVENTION (500 characters max)</b>	
SELF NANOEMULSIFYING OILY FORMULATION FOR THE ADMINISTRATION OF POORLY SOLUBLE DRUGS	
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<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.	<b>FILING FEE Amount (\$)</b> <div style="border: 1px solid black; padding: 10px; text-align: center;">\$ 160.00</div>
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Respectfully submitted,

[Page 1 of 2]

Date August 29, 2003

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Docket Number: 13961.0001

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**Docket Number** 13961.0001

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**[Page 2 of 2]**

Number 1 of 1

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**APPLICATION UNDER UNITED STATES PATENT LAWS**

**Invention:** SELF NANOEMULSIFYING OILY FORMULATION FOR THE  
ADMINISTRATION OF POORLY SOLUBLE DRUGS

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**This is a:**

- ☒ [X] Provisional Application
- ☐ [ ] Regular Utility Application
- ☐ [ ] Continuing Prosecution Application
- ☐ [ ] PCT National Phase Application
- ☐ [ ] Design Application
- ☐ [ ] Reissue Application
- ☐ [ ] Plant Application

## Self nanoemulsifying oily formulation for the administration of poorly soluble drugs

5       The present invention relates to a pharmaceutical excipient formulation, more particularly to a pharmaceutical excipient composition consisting in a self-nanoemulsifying oily formulation (SNEOF) enhancing the absorption of poorly water soluble drugs, particularly the oral absorption of taxoids and cytotoxic agents, based on improved dissolution and absorption of the drug; and providing a  
10   dose-AUC linear pharmacokinetic of the drug.

      The clinical use of some drugs is only possible if a specific drug delivery system is developed to transport them to their therapeutic target in the human body. This problem is particularly critical for water insoluble or poorly water soluble compounds for which direct injections may be impossible or problematic.

15       A few examples of therapeutic substances, which are poorly hydrosoluble, are the following: Palmitoyl Rhizoxin, Penclomedine, Vitamin A and its derivatives (retinoic acid, isotretinoin, etc.), Tamoxifen, Etoposide, Camptothecin, Navelbine, Valproic acid, Tacrolimus, Sirolimus (Rapamycin), Cyclosporin A, Clarithromycin, Testosterone, Estradiol, Progesterone,  
20   Ciprofloxacin, Fenofibrate, Benzafibrate, Azithromycin, Itraconazole, Miconazole, Propofol, Brimonidine, Latanoprost, and Paclitaxel.

      Paclitaxel, one of the best known taxoids, disrupts tubulin dynamics. It has a significant clinical activity against a broad range of tumor types including breast, lung, head and neck, bladder, and platinum-refractory ovarian carcinoma  
25   (E. K. Rowinsky. The development and clinical utility of the taxoid class of antimicrotubule chemotherapy agents. *Annu Rev Med.* 48: 353-74 (1997)). However, paclitaxel has a low therapeutic index. It is a complex diterpenoid product, with a bulky, extended fused ring system as well as a number of hydrophobic substituents, which lead to its poor solubility in water (1 µg/ml)  
30   resulting in serious formulation problems (R. T. Liggins, W. L. Hunter, H. M. Burt. Solid-state characterization of paclitaxel. *J Pharm Sci.* 86: 1458-63 (1997)).

It is highly lyophobic and the solubility of paclitaxel in lipophilic solvents, such as soybean oil is quite low and precludes the use of simple oil-in-water emulsions for formulation considerations. The commercially available product, Taxol®, is currently formulated for systemic administration in a mixture of ethanol and polyoxyethylated castor oil (Cremophor EL); the latter appears to be primarily responsible for drug related hypersensitivity reactions, rather than the drug itself (R. E. Gregory, A. F. De Lisa. Paclitaxel: a new antineoplastic agent for refractory ovarian cancer. *Clin Pharm.* 12: 401-15 (1993)). Moreover, polyoxyethylated castor oil also causes the nonlinear pharmacokinetic behavior of paclitaxel (A. Sparreboom, O. van Tellingen, W. J. Nooijen, J.H. Beijnen. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. *Cancer Res.* 56: 2112-5 (1996); O. van Tellingen, M. T. Huizing, V. R. Panday, J. H. Schellens, W. J. Nooijen, J. H. Beijnen. Cremophor EL causes (pseudo-) non-linear pharmacokinetics of paclitaxel in patients. *Br J Cancer* 81: 330-5 (1999)).

The current approaches for reducing the side effects of the actual commercial product are mainly focused on developing formulations that are devoid of polyoxyethylated castor oil. Several attempts have been made to deliver paclitaxel using alternative systems, such as nanoparticles (R. Cavalli, O. Caputo, M. R. Gasco. Preparation and characterization of solid lipid nanospheres containing paclitaxel. *Eur J Pharm Sci.* 10: 305-9 (2000); S. S. Feng, G. F. Huang, L. Mu. Nanospheres of biodegradable polymers: a system for clinical administration of an anticancer drug paclitaxel (Taxol). [In Process Citation]. *Ann Acad Med Singapore.* 29: 633-9 (2000)), liposomes (P. Crosasso, M. Ceruti, P. Brusa, S. Arpicco, F. Dosio, L. Cattel. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. *J Controlled Release.* 63: 19-30 (2000); A. Sharma, R. M. Straubinger. Novel taxol formulations: preparation and characterization of taxol-containing liposomes. *Pharm Res.* 11: 889-96 (1994)), water-soluble prodrugs (J. M. Terwogt, B. Nuijen, W. W. T. B. Huinink, J. H. Beijnen. Alternative formulations of paclitaxel. *Cancer Treat Rev.* 23: 87-95 (1997); A. Pendri, C. D. Conover, R. B. Greenwald. Antitumor activity

of paclitaxel-2'-glycinate conjugated to poly(ethylene glycol): a water-soluble prodrug. *Anticancer Drug Des.* 13: 387-95 (1998)), emulsions (P. P. Constantinides, K. J. Lambert, A. K. Tustian, B. Schneider, S. Lalji, W. Ma, B. Wentzel, D. Kessler, D. Worah, and S. C. Quay. Formulation development and antitumor activity of a filter-sterilizable emulsion of paclitaxel. *Pharm Res.* 17: 175-82 (2000); B. B. Lundberg. A submicron lipid emulsion coated with amphipathic polyethylene glycol for parenteral administration of paclitaxel (Taxol®). *J Pharm Pharmacol.* 49: 16-21 (1997); P. Kan, Z. B. Chen, C. J. Lee, I. M. Chu. Development of nonionic surfactant/phospholipid o/w emulsion as a paclitaxel delivery system. *J Controlled Release.* 58: 271-8 (1999), P. Simamora, R. M. Dannenfelser, S. E. Tabibi, S. H. Yalkowsky. Emulsion formulations for intravenous administration of paclitaxel. *PDA J Pharm Sci Technol.* 52: 170-2 (1998)) and microspheres (R. T. Liggins, S. D'Amours, J. S. Demetrick, L. S. Machan, H. M. Burt. Paclitaxel loaded poly(L-lactic acid) microspheres for the prevention of intraperitoneal carcinomatosis after a surgical repair and tumor cell spill [In Process Citation]. *Biomaterials.* 21: 1959-69 (2000); Y. M. Wang, H. Sato, I. Adachi, I. Horikoshi. Preparation and characterization of poly(lactic-co-glycolic acid) microspheres for targeted delivery of a novel anticancer agent, taxol. *Chem Pharm Bull (Tokyo).* 44: 1935-40 (1996)). However, the success is for the moment still limited. None of these alternatives has reached the stage of replacing polyoxyethylated castor oil based vehicle in the clinical application.

Another approach to overcome the hypersensitivity reactions resulting from polyoxyethylated castor oil can be the design of oral formulations of paclitaxel (J. M. M. Terwogt, M. M. Malingre, J. H. Beijnen, W. W. B. Huinink, H. Rosing, F. J. Koopman, O. van Tellingen, M. Swart, and J. H. M. Schellens. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res.* 5: 3379-84 (1999)). Oral administration of paclitaxel would, thus, prevent the adverse effects caused by the vehicle substance polyoxyethylated castor oil and offer additional advantages over intravenous administration, including elimination of the need for frequent visits to the outpatient clinic and easier chronic administration (R. T. Dorr. Pharmacology and toxicology of

Cremophor EL diluent. *Ann Pharmacother.* 28: S11-4 (1994); A. J. ten Tije, J. Verweij, W. J. Loos, and A. Sparreboom. Pharmacological effects of formulation vehicles : implications for cancer chemotherapy. *Clin Pharmacokinet* 42: 665-85 (2003)). However, preclinical studies have suggested that paclitaxel is not significantly absorbed after oral administration; the systemic bioavailability in humans after oral paclitaxel administration is less than 6% (J. M. M. Terwogt, M. M. Malingre, J. H. Beijnen, W. W. B. Huinink, H. Rosing, F. J. Koopman, O. van Tellingen, M. Swart, and J. H. M. Schellens. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res.* 5: 3379-84 (1999)). The explanations proposed to account for the poor oral bioavailability of paclitaxel are multifactorial. The most likely explanations are its affinity for the membrane-bound drug efflux pump P-glycoprotein (P-gp), metabolism by cytochromes P450 (2C8 and 3A4) and poor water solubility (R. T. Liggins, W. L. Hunter, H. M. Burt. Solid-state characterization of paclitaxel. *J Pharm Sci.* 86: 1458-63 (1997); J. van Asperen, O. van Tellingen, A. Sparreboom, A. H. Schinkel, P. Borst, W. J. Nooijen, and J. H. Beijnen. Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. *Br J Cancer.* 76: 1181-3 (1997); C. D. Britten, S. D. Baker, L. J. Denis, T. Johnson, R. Drengler, L. L. Siu, K. Duchin, J. Kuhn, and E. K. Rowinsky. Oral paclitaxel and concurrent cyclosporin A: targeting clinically relevant systemic exposure to paclitaxel. *Clin Cancer Res.* 6: 3459-68 (2000)). Moreover, the polyethoxylated castor oil (Cremophor EL) was shown to be in part responsible of the low bioavailability and poor pharmacokinetic linearity of orally administered Taxol® (H. A. Bardelmeijer, M. Ouwehand, M. M. Malingre, J. H. Schellens, J. H. Beijnen, and O. van Tellingen. Entrapment by Cremophor EL decreases the absorption of paclitaxel from the gut. *Cancer Chemother Pharmacol* 49: 119-125 (2002); M. M. Malingre, J. H. Schellens, O. Van Tellingen, M. Ouwehand, H. A. Bardelmeijer, H. Rosing, F. J. Koopman, M. E. Schot, W. W. Ten Bokkel Huinink, and J. H. Beijnen. The co-solvent Cremophor EL limits absorption of orally administered paclitaxel in cancer patients. *Br J Cancer* 85: 1472-1477 (2001)).

A number of studies have been carried out to verify in both animals and patients if the oral bioavailability of paclitaxel could be improved when the drug is administered with P-gp or cytochrome P450 inhibitors (R. T. Dorr. Pharmacology and toxicology of Cremophor EL diluent. *Ann Pharmacother.* 28: S11-4 (1994); J. van Asperen, O. van Tellingen, A. Sparreboom, A. H. Schinkel, P. Borst, W. J. Nooijen, and J. H. Beijnen. Enhanced oral bioavailability of paclitaxel in mice treated with the P-glycoprotein blocker SDZ PSC 833. *Br J Cancer.* 76: 1181-3 (1997); C. D. Britten, S. D. Baker, L. J. Denis, T. Johnson, R. Drengler, L. L. Siu, K. Duchin, J. Kuhn, and E. K. Rowinsky. Oral paclitaxel and concurrent cyclosporin A: targeting clinically relevant systemic exposure to paclitaxel. *Clin Cancer Res.* 6: 3459-68 (2000)). Cyclosporine A (CsA), a well-known immunosuppressive agent, was shown to be one of the most promising P-gp inhibitors to enhance the oral absorption of paclitaxel (J. M. M. Terwogt, M. M. Malingre, J. H. Beijnen, W. W. B. Huinink, H. Rosing, F. J. Koopman, O. van Tellingen, M. Swart, and J. H. M. Schellens. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res.* 5: 3379-84 (1999); C. D. Britten, S. D. Baker, L. J. Denis, T. Johnson, R. Drengler, L. L. Siu, K. Duchin, J. Kuhn, and E. K. Rowinsky. Oral paclitaxel and concurrent cyclosporin A: targeting clinically relevant systemic exposure to paclitaxel. *Clin Cancer Res.* 6: 3459-68 (2000)). CsA is a registered drug and thus is more readily available for clinical studies. The concomitant use of cyclosporin A for oral Taxol administration led to an increased AUC of paclitaxel (bioavailability of 20%). Nevertheless, this AUC enhancement was only reached for low doses. On the contrary, at higher doses administration showed a non linear pharmacokinetic in both rodents and human. A five fold increase (from 60 to 300mg/m<sup>2</sup>) of the dose in human only led to a 2 fold increase of plasmatic AUC (M. M. Malingre, J. M. Terwogt, J. H. Beijnen, H. Rosing, F. J. Koopman, O. van Tellingen, K. Duchin, W. W. Huinink, M. Swart, J. Lieverst, and J. H. Schellens. Phase I and pharmacokinetic study of oral paclitaxel. *J Clin Oncol* 18: 2468-2475. (2000)). This non linear dose-AUC relationship is a significant obstacle to the use of oral Taxol®. Moreover, oral Taxol® exhibited a poor tolerability in patients and

occasioned acute gastro-intestinal disorders such as nausea and vomiting. The formulation contains a high amount of ethanol (50%) and high clinically required doses lead to a significant amount of ingested ethanol. Moreover, the formulation is very bitter due to the presence of Cremophor EL. In conclusions, the oral administration of Taxol® is greatly limited by the bad tolerability after ingestion. The non linear pharmacokinetic lead the clinician to investigate high doses of Taxol® ( $> 300\text{mg/m}^2$  paclitaxel) (M. M. Malingre, J. M. Terwogt, J. H. Beijnen, H. Rosing, F. J. Koopman, O. van Tellingen, K. Duchin, W. W. Huinink, M. Swart, J. Lieverst, and J. H. Schellens. Phase I and pharmacokinetic study of oral paclitaxel. *J Clin Oncol* 18: 2468-2475. (2000)). In a phase II trial of weekly oral paclitaxel plus cyclosporine in patients with advanced non-small-cell lung cancer, interpatient variability was calculated at 40 to 45% and intra-individual variability at 15% (C. M. Kruijtzer, J. H. Schellens, J. Mezger, M. E. Scheulen, U. Keilholz, J. H. Beijnen, H. Rosing, R. A. Mathot, S. Marcus, H. van Tinteren, and P. Baas. Phase II and pharmacologic study of weekly oral paclitaxel plus cyclosporine in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 20: 4508-16 (2002)). Those points also represent important Taxol® limitations in the oral paclitaxel treatment.

Recently, it was reported that self-emulsifying oily formulation (SEOF) consisting of isotropic mixtures of oil and surfactants could significantly improve the oral availability of poorly absorbed, hydrophobic and/or lipophilic drugs (T. Gershanik, S. Benita. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *Eur J Pharm Biopharm.* 50: 179-88 (2000)). SEOFs are composed of natural or synthetic oils, surfactants and one or more hydrophilic solvents and co-solvents. The principal characteristic of SEOFs is their ability to form fine oil-in-water emulsions or microemulsions upon mild agitation following dilution by aqueous phases. These formulations can disperse in the gastrointestinal lumen to form microemulsions or fine emulsions, upon dilution with gastrointestinal fluids. In *in-vivo* absorption studies in non-fasting dogs, SEOFs elicited at least a three-fold greater  $C_{\text{max}}$  and AUC of a lipophilic naphthalene derivative than that of the drug in any other dosage form (N. H. Shah,

- M. T. Carvajal, C. I. Patel, M. H. Infeld, A. W. Malick. Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving *in vitro* dissolution and oral absorption of lipophilic drugs. *Int J Pharm.* 106: 15-23 (1994)). The absorption of ontazolast in rats was significantly enhanced by all lipid-based formulations (D. J. Hauss, S. E. Fogal, J. V. Ficorilli, C. A. Price, T. Roy, A. A. Jayaraj, and J. J. Kierns. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. *J Pharm Sci.* 87: 164-9 (1998)). Microemulsions have successfully been used to improve drug solubilization/dissolution and/or intestinal absorption of poorly absorbed drugs including CsA (P. P. Constantinides. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm Res.* 12: 1561-72 (1995); S. Tenjarla. Microemulsions: an overview and pharmaceutical applications. *Crit Rev Ther Drug Carrier Syst.* 16: 461-521 (1999)).
- Traditional surfactants are known to entrap lyophobic drugs. For example, addition of Cremophor EL to the formulation of oral drug preparations resulted in significantly diminished drug uptake and reduced circulating concentrations. The drawbacks presented by the presence of Cremophor EL or Tween 80 in drug formulations have instigated extensive research to develop alternative delivery forms. Currently, several strategies are in progress to develop Tween 80 and Cremophor EL-free formulations of docetaxel and paclitaxel, which are based on pharmaceutical, chemical or biological strategies (A. J. ten Tije, J. Verweij, W. J. Loos, and A. Sparreboom. Pharmacological effects of formulation vehicles: implications for cancer chemotherapy. *Clin Pharmacokinet* 42: 665-85 (2003); H. A. Bardelmeijer, M. Ouwehand, M. M. Malingre, J. H. Schellens, J. H. Beijnen, and O. van Tellingen. Entrapment by Cremophor EL decreases the absorption of paclitaxel from the gut. *Cancer Chemother Pharmacol* 49: 119-125 (2002)).

The rationale of a self-emulsifying oily formulation for the administration of oral paclitaxel lies in the better solubilization and absorption of paclitaxel and concomitant bioavailability variability reduction.

There is a continuing need for taxane compositions and formulations which provide a more efficient means of administering taxanes without causing undesired side effects and which have improved stability and longer shelf life.

An object of the instant invention is a pharmaceutical composition in a  
 5 form of an anhydrous self-nanoemulsifying oily formulation comprising:

- one or more therapeutic agent(s) which have low solubility in water or are water-insoluble,
- vitamin E,
- one co-solvent selected from propylene glycol and ethanol and  
 10 mixture thereof
- one surfactant selected from TPGS and tyloxapol and mixture thereof and optionally,
- a bioenhancer.

In a specific aspect of the instant invention, the pH of the composition  
 15 can be reduced to further improve the stability of the therapeutic agent. In some embodiments this is accomplished by the addition of an acidic pH adjuster which is selected from the group comprising ascorbic acid, citric acid, tartaric acid, lactic acid, oxalic acid, formic acid, benzene sulphonic acid, benzoic acid, maleic acid, glutamic acid, succinic acid, aspartic acid, diatrizoic acid, and acetic acid. The  
 20 acidifying agent may also be an inorganic acid, including, but not limited to, hydrochloric acid, sulphuric acid, phosphoric acid, and nitric acid. An anhydrous organic acid, like anhydrous citric acid, may preferably be used in the composition.

In another specific embodiment of the pharmaceutical composition  
 25 vitamin E is from 2 to 6% (w/w) of the final composition.

According to the invention, the one or more therapeutic agent(s) is selected from the group comprising anti-fungal drugs, anti-viral drugs, antibiotic drugs, anti-inflammatory drugs, anti-cancer drugs, analgesics, antidepressants, antipsychotics, hormones, antacids, coronary vasodilators, cerebral vasodilators,  
 30 psychotropics, antineoplastics, stimulants, anti-histamines, vasodilators, anti-arrhythmics, anti-hypertensive drugs, vasoconstrictors, anti-migraine drugs, anti-

coagulants and anti-thrombotic drugs, anti-pyretics, hypnotics, sedatives, anticonvulsants, anti-epileptics, neuromuscular drugs, drugs acting on Central Nervous System, hyper- and hypoglycemic agents, diuretics, anti-obesity drugs, anabolic drugs, anti-uricemic drugs and combinations thereof.

5           In a specific embodiment, the anti-cancer drug is a taxoid, preferably selected from paclitaxel, docetaxel, their derivatives, analogs and prodrugs.

When the taxoid is paclitaxel, it is present in a relative proportion between 0.5 and 4% (w/w) of the final composition, preferably between 1.5 and 3% (w/w).

10           According to one specific embodiment of the invention, preferred pharmaceutical composition for oral use comprises an emulsion including vitamin E, D- $\alpha$ -tocopheryl polyethylene glycol succinate 1000 (TPGS), tyloxapol and at least, one therapeutic agent .

15           The relative proportions of vitamin E, TPGS and tyloxapol may be respectively 2-6, 5-60 and 5-70 (w/w) of the final composition, preferably respectively 3-5, 20-40 and 20-40%.

When the composition is used for intravenous route it contains no TPGS.

20           According to another specific embodiment of the invention, the relative proportion of propylene glycol is in the range of 0-50% (w/w) of the final composition, preferably equal to 20% (w/w) and the relative proportion of ethanol is in the range of 5-50% (w/w) of the final composition, preferably equal to 30% (w/w).

25           According to the instant invention, the enhancer is one well-known from the man skilled in the art. It is advantageously selected from the group comprising cytochrome P450 2C8 inhibitors, cytochrome P450 3A4 inhibitors, multidrug resistance inhibitors, Pgp inhibitors or non specific inhibitors.

In a specific embodiment, the enhancer is selected from cyclosporine A, its analogs and derivatives.

30           The compositions according to the invention may be associated with any pharmaceutical excipient to form a dosage form, which can be administered to animals or humans via intravascular, oral, intramuscular, cutaneous and

subcutaneous routes. Specifically emulsions according to the invention can be given by any of the following routes among others: intra-abdominal, intra-arterial, intra-articular, intra-capsular, intra-cervical, intra-cranial, intra-ductal, intra-dural, intra-lesional, intra-ocular, intra-ocular, intra-lumbar, intra-mural, intra-operative, 5 intra-parietal, intra-peritoneal, intra-plural, intra-pulmonary, intra-spinal, intra-thoracic, intra-tracheal, intra-tympanic, intra-uterine, intra-ventricular, intra-venous or transdermal or can be nebulised using suitable aerosol propellants.

Self-emulsifying systems give emulsions upon dilution in aqueous media. The presence of an oily core in emulsion droplets (nonexistent in micelles) 10 enables to dissolve higher quantity of drugs and provides an encapsulation effect of stabilization. The presence of an oily core (droplets instead of micelles) enables to load bigger quantity of drugs (e.g. paclitaxel) within the SNEOF than traditional micelles-forming systems such as Taxol®. Furthermore, at equivalent drug concentration, the emulsion provides a better stability of paclitaxel 15 (degradation and/or precipitation) than micelles. The self-nanoemulsifying oily formulations give nanoemulsions with droplet size smaller than or equal to 10 nanometers. Incorporation of drugs such as paclitaxel or cyclosporin A within the SNEOFs does not significantly alter the emulsion size nor the self-nanoemulsification process. The surface offered to drug release and absorption is 20 huge and represents more than 600 m<sup>2</sup> for 1 ml of SNEOF.

The compositions according to the invention have been specifically designed to ensure self-nanoemulsification (viscosity, HLB) and to provide the best drug solubilization properties. Moreover, the surfactant choice was conditioned by the ability to release the drug so as to ensure a linear 25 pharmacokinetic even after oral administration.

In the sense of the present invention, when the pharmacokinetic is linear, the dose of therapeutic agent is proportional to the blood plasma level of the therapeutic agent desired.

Thus, two novel polymeric surfactants were used concomitantly with an 30 oily carrier: tyloxapol (oxyethylated tert-octylphenol formaldehyde polymer) and alpha-tocophéryl polyethylene glycol 1000 succinate (TPGS). Their unique

combination in SNEOF ensures a linear release of lyophobic drugs and a linear pharmacokinetic even at high doses.

An other object of the invention is the use of TPGS and tyloxapol for preparing pharmaceutical composition in the form of anhydrous self-nanoemulsifying oily formulation having linear pharmacokinetic even at high doses after oral administration.

The advantages of paclitaxel SNEOF according to the instant invention over orally given Taxol® are:

- absence of Cremophor EL,
- better taste, less bitterness,
- 2.5 to 5 fold bigger Paclitaxel content,
- 4 to 8 fold less ethanol at equivalent dose and
- 1.25 to 2.5 fold less ingested volume.

Even low paclitaxel doses of 60 or 90 mg/m<sup>2</sup> with oral Taxol® lead to bad patient tolerability (nausea, vomiting) (C. M. Kruijtzer, H. Boot, J. H. Beijnen, H. L. Lochs, F. X. Parnis, A. S. Planting, J. M. Pelgrims, R. Williams, R. A. Mathot, H. Rosing, M. E. Schot, H. Van Tinteren, and J. H. Schellens. Weekly oral paclitaxel as first-line treatment in patients with advanced gastric cancer. *Ann Oncol* 14: 197-204 (2003)). A better tolerability after ingestion is assumed based on a significative reduction of the ethanol content and of the bitterness reduction. Furthermore, selected excipients (TPGS, tyloxapol) have a lower rodent oral DL50 than Cremophor EL. The reduced volume and reduced overall toxicity could safely allow to administrate higher paclitaxel dose to patients than oral Taxol® formulation for which the maximal tolerated dose (MTD) was estimated at 360mg/m<sup>2</sup> (M. M. Malingre, J. M. Terwogt, J. H. Beijnen, H. Rosing, F. J. Koopman, O. van Tellingen, K. Duchin, W. W. Huinink, M. Swart, J. Lieverst, and J. H. Schellens. Phase I and pharmacokinetic study of oral paclitaxel. *J Clin Oncol* 18: 2468-2475. (2000)). Moreover the dose-AUC non-linearity and pharmacokinetic variabilities of orally given Taxol® constitute the main limitations to an oral clinical practice use and security (M. M. Malingre, J. H. Beijnen, H. Rosing, F. J. Koopman, O. van Tellingen, K. Duchin, W. W. Ten

Bokkel Huinink, M. Swart, J. Lieverst, and J. H. Schellens. A phase I and pharmacokinetic study of bi-daily dosing of oral paclitaxel in combination with cyclosporin A. *Cancer Chemother Pharmacol* 47: 347-54 (2001)).

The self-nanoemulsifying compositions according to the instant invention  
 5 may be used for the treatment of different diseases like cancers, tumours, Kaposi's sarcoma, malignancies, uncontrolled tissue or cellular proliferation secondary to tissue injury, and any other disease conditions responsive to taxoids such as paclitaxel and docetaxel, and/or prodrugs and derivatives of the foregoing. Among  
 10 the types of carcinoma which may be treated particularly effectively with oral paclitaxel, docetaxel, other taxoids, and their prodrugs and derivatives, are hepatocellular carcinoma and liver metastases, cancers of the gastrointestinal tract, pancreas, prostate and lung, and Kaposi's sarcoma. Examples of non-cancerous disease conditions which may be effectively treated with these active agents administered orally in accordance with the present invention are uncontrolled  
 15 tissue or cellular proliferation secondary to tissue injury, polycystic kidney disease, inflammatory diseases (e. g., arthritis) and malaria.

The novel compositions may be administered in any known pharmaceutical oral dosage form. For example, the formulations may be encapsulated in a soft or hard gelatin capsule or may be administered in the form  
 20 of a liquid oily preparation. Each dosage form may include, apart from the essential components of the composition conventional pharmaceutical excipients, diluents, sweeteners, flavouring agents, colouring agents and any other inert ingredients regularly included in dosage forms intended for oral administration (see e. g., Remington's Pharmaceutical Sciences, 17th Ed., 1985).

25 Precise amounts of each of the target drugs included in the oral dosage forms will vary depending on the age, weight, disease and condition of the patient.

Although some of the oral formulations of the invention may provide therapeutic blood levels of the taxoid active ingredient when administered alone, an advantageous method of the invention for treating mammalian patients  
 30 (particularly human patients) suffering from taxoid-responsive disease conditions is to administer the oral formulations containing the taxoid target agent

concomitantly with the administration of at least one dose of an oral bioavailability enhancing agent. This bioenhancer can be concomitantly formulated in the SNEOF or administered separately. Another advantageous method of the invention for treating mammalian patients is to administer the oral formulations containing the taxoid target agent concomitantly or separately with another antitumor agent like carboplatinum and the like.

The preferred embodiment of the method of the invention for oral administration to humans of paclitaxel, its derivatives, analogs and prodrugs, and other taxoids comprises the oral administration of an oral absorption or bioavailability enhancing agent to a human patient simultaneously with, or prior to, or both simultaneously with and prior to the oral administration to increase the quantity of absorption of the intact target agent into the bloodstream.

Different advantages of the present invention will be readily appreciated with the following figures, tables and examples.

Figure 1 illustrates the pharmacokinetic profiles of 10 mg/kg intravenously administered paclitaxel with or without 10mg/kg cyclosporin A oral pre-treatment.

Figure 2 illustrates the pharmacokinetic profiles of oral 10 mg/kg paclitaxel formulations according to the invention with or without 10 mg/kg cyclosporin A oral pre-treatment and compared to paclitaxel alone.

## **EXAMPLE 1: CHARACTERIZATION OF PACLITAXEL EMULSIONS FOLLOWING 1:10 DILUTION OF SNEOFS WITH WATER**

### **1. MATERIALS AND METHODS**

#### **1.1. Materials**

Paclitaxel (MW 853) with 99.34% (w/w) purity (HPLC) was purchased from Farmachem (Lugano, Switzerland). Vitamin E and tyloxapol were bought

from Sigma (St. Louis, MO, USA). D- $\alpha$ -tocopheryl polyethylene glycol succinate 1000 (TPGS) was a gift from Eastman Chemical (Kingsport, TN, USA). Ethanol was bought from SDS (Peypin, France). All solvents were HPLC grade

## 5 1.2. Methods

### **Preparation of paclitaxel SNEOFs**

SNEOFs were firstly prepared by successive addition and mixing of each excipient. When the oily carrier is clear and homogeneous, paclitaxel is added and  
10 quickly dissolved under mild agitation. For combined formulations, Cyclosporin A is lastly added and quickly dissolved under mild agitation in the SNEOF. In the same manner, pH-lowered formulations are obtained by addition of 0.01 or 0.02 % anhydrous citric acid in the previously prepared paclitaxel-containing SNEOF. Its dissolution is slow and requires agitation.

15 Paclitaxel emulsion may be formed by dilution of SNEOFs with distilled water.

### **Examples of paclitaxel SNEOFs**

The following compositions have been prepared according to the above-  
20 disclosed method.

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31.75
Ethanol anhydrous, absolute	30
Tyloxapol	31.75

Components	% (w, w)
Paclitaxel	3
Vitamin E	5
TPGS	31
Ethanol anhydrous, absolute	30
Tyloxapol	31

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
Ethanol anhydrous, absolute	30
Tyloxapol	63.5

#### Examples of combined formulations

The following compositions have been prepared according to the above-disclosed method.

5

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31
Ethanol anhydrous, absolute	30
Tyloxapol	31
Cyclosporin A	1.5

Components	% (w, w)
Paclitaxel	3
Vitamin E	5
TPGS	30.25
Ethanol anhydrous, absolute	30
Tyloxapol	30.25
Cyclosporin A	1.5

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
Ethanol anhydrous, absolute	30
Tyloxapol	62
Cyclosporin A	1.5

#### Examples of pH-lowered formulations

10

The following compositions have been prepared according to the above-disclosed method.

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31.75
Ethanol anhydrous, absolute	30
Tyloxapol	31.75
Citric acid	0.01

### Droplet size

Emulsions were formed following 1:10 dilution of paclitaxel SNEOF  
 5 with distilled water. The droplet size of the resulting emulsions was determined  
 by the PCS method using a Nanosizer (Malvern, UK)

### Stability Study

SNEOFs containing 1.5 and 3% (w/w) paclitaxel were prepared. A  
 10 combined form of 1.5% paclitaxel plus 1.5% cyclosporin A was also prepared.  
 The chemical stability of paclitaxel in SNEOFs was monitored using an analytical  
 HPLC method (M. Andreeva, P. D. Iedmann, L. Binder, V. W. Armstrong, H.  
 Meden, M. Binder, M. Oellerich. A simple and reliable reversed-phase high-  
 performance liquid chromatographic procedure for determination of paclitaxel  
 15 (taxol) in human serum. *Ther Drug Monit.* 19: 327-32 (1997); A. Sharma, W. D.  
 Conway, R. M. Straubinger. Reversed-phase high-performance liquid  
 chromatographic determination of taxol in mouse plasma. *J Chromatogr B*  
*Biomed Appl.* 655: 315-9 (1994)).

## 20 2. RESULTS

### 2.1. Physicochemical Characterization

#### Droplet size

25 Following 1:10 dilution of paclitaxel SNEOFs (1.5% and 3% w/w) in  
 distilled water, the droplet size of the resulting nanoemulsions was in average  
 equal to  $10 \pm 4.0$  nm with a low Polydispersity index ( $<0.15$ ).

## 2.2. Stability Study

The preliminary chemical stability studies indicated that paclitaxel in the  
5 SNEOFs was stable at 4, 25 and 40 °C. The drug content in SNEOFs at 4, 25 and  
40 °C did not change over three months.

## 10 EXAMPLE 2: PHARMACOKINETIC STUDY OF ORAL PACLITAXEL EMULSIONS AFTER CYCLOSPORIN A ORAL PRETREATMENT

### 1. MATERIALS AND METHODS

#### 15 1.1. Materials

##### 1.1.1. *Animals*

20	Strains:	FVB Wild-type mice
	Source:	Breeding stocks of the animal facility of the Netherlands Kancer Institute (NKI)
	Age:	8-14 weeks
	Body weight:	18-30 gram
	Gender:	Female
25	Housing:	Animal Department of the NKI

##### 1.1.2. *Drug*

	<b>Oral Formulation:</b>	Cyclosporin A (Sandimmun®)
30	Source:	Novartis
	Vehicle:	Cremophor EL: Ethanol (65:35, v/v)
	Concentration:	50 mg/mL
	Route:	Oral (p.o.)
	Dose:	10 mg/kg

- Oral Taxol:** Paclitaxel (Taxol®)  
**Source:** Bristol-Myers Squibb  
**Vehicle:** Cremophor EL: Ethanol (1:1; v/v)  
 5 **Concentration:** 6 mg/mL and 2mg/mL after 1:3 dilution in water for injection (WFI)  
**Route:** Oral (p.o.)  
**Dose:** 10 mg/kg
- 10 **Oral Formulation 1:** Paclitaxel SNEOF  
**Source:** Novagali SAS  
**Vehicle:** Tyloxapol / TPGS / Ethanol / Vitamin E  
**Concentration:** 15 mg/mL for SEOF and 1.5 mg/mL after 1:10 dilution in WFI  
 15 **Route:** Oral (p.o.)  
**Dose:** 10 mg/kg

Oral formulation 1 composition according to the invention

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31.75
Ethanol anhydrous, absolute	30
Tyloxapol	31.75

- 20 **Oral Formulation 2 according to the invention: Paclitaxel / Cyclosporin A SNEOF**  
**Source:** Novagali SAS  
**Vehicle:** Tyloxapol / TPGS / Ethanol / Vitamin E  
**Concentration:** Paclitaxel: 15 mg/mL for SEOF and 1.5 mg/mL  
 25 after 1:10 dilution in WFI  
 Cyclosporin A: 15 mg/mL for SEOF and 1.5 mg/mL after 1:10 dilution

Route: Oral (p.o)  
 Dose: 10 mg/kg paclitaxel and 10 mg/kg cyclosporin A

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31
Ethanol anhydrous, absolute	30
Tyloxapol	31
Cyclosporin A	1.5

Oral formulation 2 composition

5

**I.V. Formulation: Paclitaxel**

Source: Paclitaxel (pure compound) supplied by Novagali  
 Vehicle: Polysorbate 80: Ethanol (1:1, v/v)  
 Concentration: 6 mg/mL, 1.5 mg/ml after 1:4 dilution in saline  
 Route: intravenous (i.v.)  
 Dose: 10 mg/kg

10

*1.1.3. Preparation of drug solutions for oral administration*

15 Cyclosporin A (Sandimmun®) was diluted 1:25 in water for injection to yield a final concentration of 2 mg/mL. A volume of 5 µL per gram body weight was administered to the animals resulting in a dose of 10 mg/kg of cyclosporin A.

20 Paclitaxel self-nanoemulsifying oily formulations (SNEOF) n°1 and 2 were diluted 1:10 in WFI to give a microemulsion. A volume of 6.67 µL per gram (body weight) was administered to the animals resulting in a dose of 10 mg/kg of paclitaxel.

25 Paclitaxel in Cremophor EL: ethanol (Taxol®) was diluted with water for injection to a final concentration of 1.5 mg/ml (1:4 dilution). A volume of 6.67 µL per gram (body weight) was administered resulting in a dose level of 10 mg/kg of paclitaxel.

#### 1.1.4. Preparation of drug solutions for intravenous administrations

The stock solution of paclitaxel in Polysorbate 80: ethanol (1:1; v/v) was diluted 1:4 with saline to achieve a final concentration of 1.5 mg/mL. A volume of 6.67  $\mu$ L per gram (body weight) was administered to the animals, resulting in a dose level of 10 mg/kg of paclitaxel.

#### 1.2. Study set up

Cohort	CsA oral Pre-treatment	Paclitaxel vehicle	Animal number	Blood sampling times
1	10 mg/kg	TAXOL 0.6%	20	1, 2, 4 & 8 h
2	10 mg/kg	SNEOF 1.5%	20	1, 2, 4 & 8 h
3	None	SNEOF 1.5% + 1.5% CsA	20	1, 2, 4 & 8 h
4	None	PS 80: EtOH 0.6% IV	24	5 & 30 min, 1, 2, 4 & 8 h
5	10 mg/kg	PS 80: EtOH 0.6% IV	24	5 & 30 min, 1, 2, 4 & 8 h

10

Each cohort through 1 to 3 consisted of 20 Wild-type mice and cohorts. Cohort 1 received paclitaxel in the conventional formulation of Cremophor EL and ethanol at a dose of 10 mg/kg.

Cohorts 2 received a paclitaxel microemulsion formulation according to the invention at a dose of 10 mg/kg of paclitaxel.

Cohorts 1 and 2 received a pre-treatment of 10mg/kg cyclosporin A, 30 minutes prior to paclitaxel administration.

Cohort 3 received both cyclosporin and paclitaxel in a microemulsion formulation according to the instant invention at dose levels of 10 mg/kg of paclitaxel and 10 mg/kg of cyclosporin A.

Cohorts 4 and 5 are reference groups for the calculation of the bioavailability and consisted of 24 animals per cohort. They received paclitaxel at a dose of 10 mg/kg by intravenous injection in the tail vein following oral cyclosporin A or cyclosporin A vehicle.

Oral drug administrations was done by stainless steel gavage using a glass syringe (250 µl: Hamilton luer tip) or a disposable polypropylene syringe (1 ml). The gavage was inserted via the oesophagus into the stomach. Cyclosporin A was administered orally  $18 \pm 2$  min prior to oral paclitaxel. At times 1, 2, 4 and 8 h after oral paclitaxel administration, animals (n=5 per time point per group) were anaesthetized with metofane and blood was sampled by cardiac puncture.

Intravenous drug administrations were done by injection into the tail vein using a disposable polypropylene syringe (300 µl) provided with fixed 29 g needle. Cyclosporin A was administered  $30 \pm 5$  min prior to intravenous paclitaxel. At times 5, 30 min, 1, 2, 4 and 8 h after intravenous paclitaxel administration, animals (n=4 per time point per group) were anaesthetized with metofane and blood was sampled by cardiac puncture.

#### *1.2.1. Sample collection, handling and storage*

15

At the specified times, animals were anaesthetized using metofane. After fixation on their back, with their chest in an upright position blood was collected by cardiac puncture using a 1 mL polypropylene syringe fitted with 25 g needle. Blood was transferred immediately into tubes containing potassium EDTA as anticoagulant and mixed by inversion. Blood samples were centrifuged vial for 5 min at 4000 g. The supernatant plasma fraction was transferred to a clean tube with appropriate label and stored at -20°C until analyses.

#### *1.2.2. Number of animals / samples*

25

Based on previous experience, 5 animals per time point was sufficient to accurately determine the AUC of the plasma concentration-time curves after oral dosing, whereas 4 animals per time point sufficed for intravenous dose groups. Plasma samples were obtained from all animals.

30

#### *1.2.3. Analytical method*

Analyses of paclitaxel levels in the plasma samples used a validated HPLC-UV methodology (Sparreboom, A., van Tellingen, O., Nooijen, W.J., and Beijnen, J.H. Determination of paclitaxel and metabolites in mouse plasma, tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Appl*, 664: 383-391, 1995.).

#### 1.2.4. Assay performance control

Test samples were analysed for paclitaxel in singular within an analytical batch, consisting of a set of calibration standards and QC samples. Per series of 40 test samples at least 3 QC samples containing paclitaxel at concentrations over the expected range were analysed in duplicate. Results of batch analysed were accepted if:

- the correlation coefficient (r) of the calibration curve is higher than 0.98.
- at least 4 of the 6 QC samples are within  $\pm 20\%$  of their respective nominal values; 2 of the 6 QC samples (not both at the same concentration) may be outside the  $\pm 20\%$  of their respective nominal values.

20

#### 1.2.5. Data reprocessing

Chromatographic data acquisition and processing were done using a Chromeleon (v.6) chromatography data station. Calibration lines were fitted by weighed least squares regression analysis using the reciprocal of the squared concentration as the weight factor.

Plasma concentration data were reported. Plasma concentrations versus time curves were fitted using the MEDIWARE software package (version 3.0) and pharmacokinetic parameters: Area under the plasma concentration-time curve (AUC), Maximum plasma level ( $C_{max}$ ), Elimination half-life ( $t_{1/2}$ ) and Biological availability (F) were calculated.

## 2. RESULTS

They are illustrated in figures 1 and 2 and in the following table.

- 5                    Paclitaxel bioavailability of 10mg/kg paclitaxel oral formulations with or  
without 10 mg/kg oral cyclosporin A pre-medication

Cohort	CsA oral Pre- treatment	Paclitaxel vehicle	AUC (mean $\pm$ SE) (ng/ml.h)	Bioavailability (%)
1	10 mg/kg	TAXOL 0.6%	2984 $\pm$ 173	21.3
2	10 mg/kg	SNEOF 1.5%	2670 $\pm$ 249	19.1
3	None	SNEOF 1.5% + 1.5% CsA	2772 $\pm$ 344	19.8
4	None	PS 80: EtOH 0.6% IV	5961 $\pm$ 374	100
5	10 mg/kg	PS 80: EtOH 0.6% IV	14006 $\pm$ 725	100

## 3. CONCLUSION

- 10                    Cyclosporin A is known to be an oral taxanes bioenhancer. Oral pre-  
treatment with cyclosporin A lead to a significant increase of intravenously  
administered paclitaxel AUC from 5961  $\pm$  374 to 14006  $\pm$  725 (2.35 fold). Oral  
pre-treatment with cyclosporin A also lead to a significant increase of orally  
administered paclitaxel AUC (data not shown).

- 15                    At the dose of 10 mg/kg of paclitaxel plus 10 mg/kg of Cyclosporin A  
orally administered to wild type mice, both the SNEOF and the Taxol®  
formulation exhibited an equivalent bioavailability of approximately 20%.

- The paclitaxel and cyclosporin A-containing SNEOF also lead to a 20%  
bioavailability of paclitaxel. This formulation illustrates the possibility to use a  
20    combined oral dosage form (SNEOF) of the drug and a bioenhancer.

**EXAMPLE 3: DOSE-AUC PHARMACOKINETIC STUDY OF  
ORAL PACLITAXEL EMULSIONS AFTER CYCLOSPORIN A ORAL  
PRE-TREATMENT**

**5 1. MATERIALS AND METHODS**

**1.1. Materials**

***1.1.1. Animals***

10

Strains:	FVB Mdr1ab(-/-) mice
Source:	Breeding stocks of the animal facility of the NKI
Age:	8-14 weeks
Body weight:	18-30 gram
15 Gender:	Female
Housing:	Animal Department of the NKI

***1.12. Drug***

20

<b>Oral Formulation:</b>	<b>Paclitaxel SNEOF</b>
Source:	Novagali SAS
Vehicle:	Tyloxapol / TPGS / Ethanol / Vitamin E
Concentration:	<b>15 mg/mL paclitaxel</b>
Route:	Oral (p.o)
25 Dose:	<b>10 and 30 mg/kg</b>

Components	% (w, w)
Paclitaxel	1.5
Vitamin E	5
TPGS	31.75
Ethanol anhydrous, absolute	30
Tyloxapol	31.75

Composition of paclitaxel SNEOF

**I.V. Formulation: Paclitaxel**

- 5           Source:           Paclitaxel (pure compound) supplied by Novagali
- Vehicle:       Polysorbate 80: Ethanol (1:1, v/v)
- Concentration: 6 mg/mL, 1.5 mg/ml after 1:4 dilution in WFI
- Route:         intravenous (i.v.)
- Dose:          10 mg/kg

10

**1.1.3. Preparation of drug solutions for oral and intravenous administration**

Paclitaxel self-nanoemulsifying oily formulations (SNEOF) were diluted 1:10 in WFI to give a microemulsion containing 1.5 mg/ml of paclitaxel. A  
15 volume of 6.67  $\mu$ L per gram (body weight) was administered orally to the animals resulting in a dose of 10 mg/kg of paclitaxel.

Paclitaxel self-nanoemulsifying oily formulations (SNEOF) were diluted 1:10 in WFI to give a microemulsion containing 1.5 mg/ml of paclitaxel. A  
20 volume of 20  $\mu$ L per gram (body weight) was administered orally to the animals resulting in a dose of 30 mg/kg of paclitaxel.

The stock solution of paclitaxel in Polysorbate 80: ethanol (1:1; v/v) was diluted 1:4 with saline to achieve a final concentration of 1.5 mg/mL. A volume of  
25 6.67  $\mu$ L per gram (body weight) was administered intravenously to the animals, resulting in a dose level of 10 mg/kg of paclitaxel.

## 1.2. Study set up

### 1.2.1. *Plasma pharmacokinetic study*

5 Cohorts 1 and 2 (each cohort containing 25 mice) received paclitaxel at dose levels of 10 and 30 mg/kg, respectively.

Cohort 3 (containing 24 mice) received paclitaxel intravenously at a dose of 10 mg/kg. Dose levels higher than 20 mg/kg cannot be given intravenous due to acute lethal toxicity of the formulation (Polysorbate 80 and ethanol). Moreover,  
10 given that the oral bioavailability ( $F = AUC_{oral}/AUC_{i.v.}$ ) is around 35%, an intravenous dose of 10 mg/kg is expected to be a reasonable reference for calculating the oral bioavailability.

### 1.2.2. *Mass balance study*

15

Two cohorts of 5 mice were placed in metabolic cages and were allowed to accustom to these cages for two days. Subsequently, these cohorts received paclitaxel from the SNEOF formulation at dose levels of 10 and 30 mg/kg, respectively. Urine and feces were collected after every 24 h interval for up to 96  
20 h after drug administration.

Oral drug administrations were done by stainless steel gavage using a glass syringe (250  $\mu$ l: Hamilton luer tip) or a disposable polypropylene syringe (1 ml). The gavage was inserted via the oesophagus into the stomach. At time points 0.5, 1, 2, 4 and 8 h after oral paclitaxel administration, blood from the animals  
25 (n=5 per time point per group) was sampled by cardiac puncture.

Intravenous drug administrations were done by injection into the tail vein using a disposable polypropylene syringe (300  $\mu$ l) provided with fixed 29 g needle. At time points 5, 30 min, 1, 2, 4 and 8 h after intravenous paclitaxel administration, blood from the animals (n=4 per time point per group) was  
30 sampled by cardiac puncture.

### *1.2.3. Sample collection, handling and storage*

#### **Plasma pharmacokinetic study**

At the specified times, animals were anaesthetized using metofane. After  
5 fixation on their back, with their chest in an upright position blood was collected  
by cardiac puncture using a 1 mL polypropylene syringe fitted with 25 g needle.  
Blood was transferred immediately into tubes containing potassium EDTA as  
anticoagulant and mixed by inversion. Blood samples were centrifuged vial for 5  
min at 4000 g. The supernatant plasma fraction was transferred to a clean tube  
10 with appropriate label and stored at -20°C until analyses.

#### **Mass balance study**

The faeces and urine samples from one animal were pooled to yield  
faeces and urine samples containing all drug excreted by each animal during the  
15 full period of 96 h. Faeces samples were homogenized in an aqueous solution of  
bovine serum albumin (4%; w/v) using a volume of 10 ml per gram of faeces.  
Homogenates were stored at -20°C until analysis.

Urine samples were diluted 1 + 4 (v/v) with blank human plasma and  
stored at -20°C until analysis.

20

### **1.3. Number of animals / samples**

#### **Plasma pharmacokinetic study**

5 animals per time point were used to determine the AUC of the plasma  
25 concentration-time curves after oral dosing, whereas 4 animals per time point  
were used for intravenous dose groups. Plasma samples for subsequent analysis  
were obtained from all animals.

#### *1.3.1. Mass balance study*

30

10 animals were used to determine the fraction of unchanged paclitaxel excreted via the faeces and urine. The samples of each individual animal were pooled resulting in 10 faeces and 10 urine samples for subsequent analysis.

#### 5 1.4. Analytical method

Analyses of paclitaxel levels in the plasma samples were a validated HPLC – UV methodology (Sparreboom, A., van Tellingen, O., Nooijen, W.J., and Beijnen, J.H. Determination of paclitaxel and metabolites in mouse plasma, 10 tissues, urine and faeces by semi-automated reversed-phase high-performance liquid chromatography. J. Chromatogr B. Biomed Appl., 664: 383-391, 1995).

##### *1.4.1. Assay performance control*

15 Test samples were analysed for paclitaxel in singular within an analytical batch, consisting of a set of calibration standards and QC samples. Per series of 60 test samples at least 3, QC samples containing paclitaxel at concentrations over the expected range were analysed in duplicate. Results of batch analysed were accepted if:

- 20 - the correlation coefficient ( $r$ ) of the calibration curve is higher than 0.98.
  - at least 4 of the 6 QC samples are within  $\pm 20\%$  of their respective nominal values; 2 of the 6 QC samples (not both at the same concentration) may be outside the  $\pm 20\%$  of their respective nominal values.
- 25

##### *1.4.2. Data reprocessing*

Chromatographic data acquisition and processing were done using a 30 Chromeleon (v.6) chromatography data station. Calibration lines were fitted by weighed least squares regression analysis.

Plasma concentration data were reported. Plasma concentrations versus time curves were fitted using the MEDIWARE software package (version 3.0) and pharmacokinetic parameters: Area under the plasma concentration-time curve (AUC), Maximum plasma level ( $C_{max}$ ), Elimination half-life ( $t_{1/2}$ ) and Biological availability (F) were calculated.

## 2. RESULTS

They are given in the following table

Paclitaxel plasmatic AUC of paclitaxel oral formulations after 10 mg/kg oral  
cyclosporin A pre-medication

Formulation	Dose (mg/kg)	Route	Excretion (%) (mean $\pm$ SE)	AUC (ng/mL.h) (mean $\pm$ SE)
SNEOF	10	oral	27.9 $\pm$ 5.3	1934 $\pm$ 170
SNEOF	30	oral	41.3 $\pm$ 4.4	6381 $\pm$ 354

## 3. CONCLUSION

An increase of the oral paclitaxel dose from 10 to 30 mg/kg resulted in a 3-fold linear increase of the paclitaxel AUC. These results suggested that the bioavailability of oral paclitaxel was proportional to the administered SNEOF dose.

## CLAIMS

1. A pharmaceutical composition in a form of an anhydrous self-nanoemulsifying oily formulation comprising:
  - 5           - one or more therapeutic agent(s) which have low solubility in water or are water-insoluble,
  - vitamin E,
  - one co-solvent selected from propylene glycol and ethanol and mixture thereof
  - 10          - one surfactant selected from TPGS and tyloxapol and mixture thereof and optionally,
  - a bioenhancer.
2. A pharmaceutical composition according to claim 1 further comprising an  
15   acidic pH adjuster.
3. A pharmaceutical composition according to anyone of claims 1 to 2, wherein vitamin E is from 2 to 6% (w/w) of the final composition.
- 20   4. A pharmaceutical composition according to anyone of claims 1 to 3, wherein the one or more therapeutic agent(s) is selected from the group comprising anti-fungal drugs, anti-viral drugs, antibiotic drugs, anti-inflammatory drugs, anti-cancer drugs, analgesics, antidepressants, antipsychotics, hormones, antacids, coronary vasodilators, cerebral vasodilators, psychotropics, antineoplastics,  
25   stimulants, anti-histamines, vasodilators, anti-arrythmics, anti-hypertensive drugs, vasoconstrictors, anti-migraine drugs, anti-coagulants and anti-thrombotic drugs, anti-pyretics, hypnotics, sedatives, anticonvulsants, anti-epileptics, neuromuscular drugs, drugs acting on Central Nervous System, hyper- and hypoglycemic agents, diuretics, anti-obesity drugs, anabolic drugs, anti-uricemic drugs and  
30   combinations thereof .

5. A pharmaceutical composition according to anyone of claims 1 to 4, wherein the anti-cancer drug is a taxoid, preferably selected from paclitaxel, docetaxel, their derivatives, analogs and prodrugs.
- 5 6. A pharmaceutical composition according to anyone of claims 1 to 5, wherein the taxoid is paclitaxel in a relative proportion between 0.5 and 4% (w/w) of the final composition, preferably between 1.5 and 3% (w/w).
7. A pharmaceutical composition according to anyone of claims 1 to 6, wherein  
10 the relative proportions of vitamin E, TPGS and tyloxapol are respectively 2-6, 5-60 and 5-70 (w/w) of the final composition, preferably respectively 3-5, 20-40 and 20-40%.
8. A pharmaceutical composition according to anyone of claim 1 to 7 wherein the  
15 relative proportion of propylene glycol is in the range of 0-50% (w/w) of the final composition, preferably equal to 20% (w/w) and the relative proportion of ethanol is in the range of 5-50% (w/w) of the final composition, preferably equal to 30% (w/w).
- 20 9. A pharmaceutical composition according to anyone of claims 1 to 8, wherein the enhancer is selected from the group comprising cytochrome P450 2C8 inhibitors, cytochrome P450 3A4 inhibitors, multidrug resistance inhibitors, Pgp inhibitors or non specific inhibitors.
- 25 10. A pharmaceutical composition according to claim 9, wherein the enhancer is cyclosporine A, its analogs and derivatives.
11. A pharmaceutical composition according to anyone of claims 2 to 10, wherein the acidic pH adjuster is anhydrous citric acid.

12. A pharmaceutical dosage form comprising an anhydrous self-nanoemulsifying oily formulation composition according to anyone of claims 1 to 11 associated to suitable pharmaceutical excipients.

5 13. A pharmaceutical dosage form according to claim 12, which is suitable for the oral route.

14. A pharmaceutical dosage form according to claim 13 wherein the composition is encapsulated in a soft or hard gelatin capsule or is a liquid oily preparation.

10

15. A pharmaceutical dosage form according to claim 12, which is suitable for the intravenous route.

16. Use of an anhydrous self-nanoemulsifying oily formulation according to  
15 anyone of claims 1 to 11 for the manufacture of a medicament useful in the treatment of taxoid-responsive diseases.

17. Use according to claim 16 for administration to patients receiving  
simultaneously with, concomitantly or prior to, bioavailability enhancing agent  
20 and/or another antitumor agent.

18. Use of an anhydrous self-nanoemulsifying oily formulation according to  
anyone of claims 1 to 11 for the manufacture of a medicament wherein the dose of  
the therapeutic agent administered is linearly proportional to the blood plasma  
25 level of the therapeutic agent desired.

19. Use of TPGS and tyloxapol for preparing pharmaceutical composition in the  
form of anhydrous self-nanoemulsifying oily formulation suitable for preparing a  
medicament wherein the dose of the therapeutic agent administered is linearly  
30 proportional to the blood plasma level of the therapeutic agent desired.

**ABSTRACT**

5 A pharmaceutical composition in a form of an anhydrous self-nanoemulsifying  
oily formulation comprising:

- one or more therapeutic agent(s) which have low solubility in water  
or are water-insoluble,
- vitamin E,
- 10 - one co-solvent selected from propylene glycol and ethanol and  
mixture thereof
- one surfactant selected from TPGS and tyloxapol and mixture thereof  
and optionally,
- a bioenhancer.

Figure 1

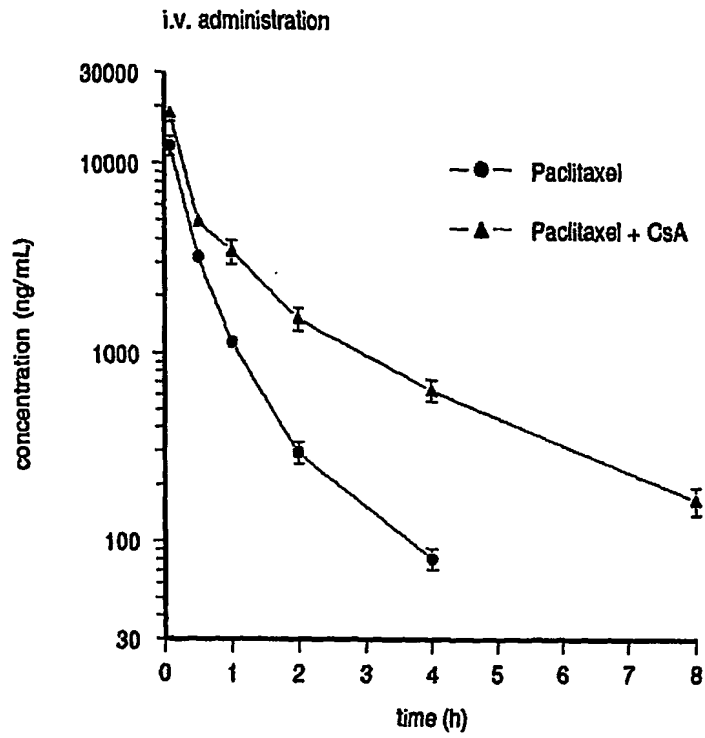
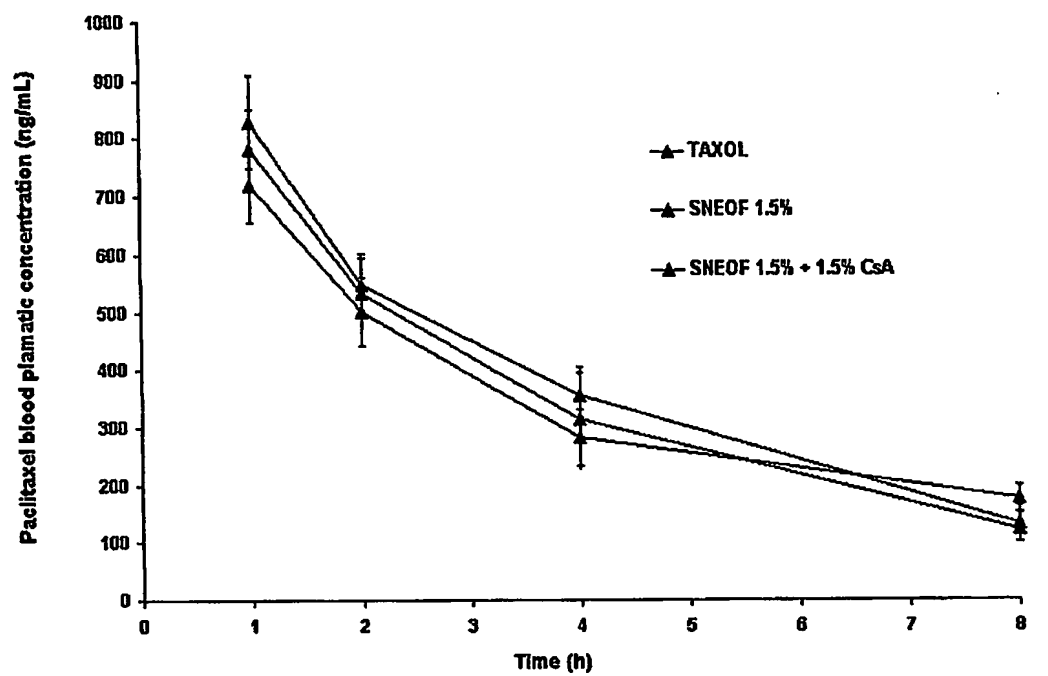


Figure 2



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